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Diet Selection by Steers Using Microhistological and Stable Carbon Isotope Ratio Analyses¹

L. L. Bennett*, A. C. Hammond †,2 , M. J. Williams †,3 , C. C. Chase, Jr. † , and W. E. Kunkle*

*University of Florida, Gainesville 32611-0910 and †ARS, USDA, Subtropical Agricultural Research Station, Brooksville, FL 34601-4672

ABSTRACT: Two methods of determining diet botanical composition, microhistological (MH), and stable carbon isotope ratio (CR) analyses were used to determine botanical composition of ingesta and fecal grab samples in steers grazing rhizoma peanut-mixed tropical grass pastures. Three pastures were used over two grazing seasons, 1992 and 1993, in Brooksville, FL. A weighted-disc double-sampling technique was used to determine forage mass and botanical composition, percentage of rhizoma peanut (Arachis glabrata), grass (Paspalum notatum and Cynodon dactlyon), and forb (primarily Chenopodium ambrosioides) on offer every 28 d throughout the grazing seasons. There was an effect of sampling date (P < .001), sampling date \times pasture (P < .001), and sampling date \times year (P < .001) on forage mass on offer. There was a pasture \times year \times sampling date interaction (P < .001) for all botanical components. In 1992 and 1993, using cannulated steers sampled every 56 d, there were interactions with year for rhizoma peanut and forb (P < .05), but not for grass with MH analysis (components: rhizoma peanut, grass, and forb). Ingesta and fecal rhizoma peanut (r = .73 and .92 for 1992 and 1993, respectively) and ingesta and fecal forb (r = .86 and .98 for 1992 and 1993, respectively) were positively correlated (P < .001). Ingesta and fecal grass were positively correlated (r = .52, P < .001), but the correlation was not as high. With the CR analysis (components: Calvin cycle $[C_3]$ plants and C_4 -dicarboxylic acid pathway $[C_4]$ plants), ingesta and corrected fecal (corrected for in vitro organic matter digestibility [IVOMD]) C3 plants were positively correlated (r = .62; P < .001). Diet composition of fecal grab samples from noncannulated steers, collected on the same sampling schedule as for hand-clipped pasture samples, differed at times due to the complexity of the sward (both rhizoma peanut and forb constituted a single component, C3, in the CR analysis). Based on these results, if there is a substantial contribution of forb to the diet, fecal microhistological analysis may be more informative than fecal carbon ratio analysis for estimating diet selection by cattle grazing tropical pastures.

Key Words: Botanical Composition, Cattle, Feeding Behavior, Foraging, Intake, Mixed Pastures

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Introduction

Cattle grazing mixed swards have the potential to selectively graze different forage species. Cattle generally consume forage of higher nutritional quality than that of total forage available (Coleman and Barth, 1973; Moore et al., 1987).

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Both microhistological (MH) and stable carbon isotope ratio (CR) analyses have been used to determine botanical composition of mixed swards and diets of grazing cattle. The basis for MH analysis of fecal, ruminal, esophageal, or hand-clipped samples is that different plant species have different epidermal characteristics (Sparks and Malechek, 1968). Stable carbon isotope ratio analysis is based on differential assimilation of ¹²C and ¹³C during photosynthesis (Minson et al., 1975). Plants that fix carbon dioxide through the Calvin cycle (C₃) have different stable carbon isotope ratios than those that utilize the C₄dicarboxylic acid pathway (C_4). The ratio of natural carbon isotopes occurring in the diet, feces, and ruminal contents has been used to determine the proportion of legumes (C3 species) and tropical grasses (C₄ species) selected by ruminants (Jones et al., 1979).

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²Current address: USDA, ARS, PWA, 800 Buchanan St., Albany, CA 94710.

 $^{^3\}mbox{To}$ whom correspondence should be addressed: 22271 Chinsegut Hill $\,$ Rd.

The objective of this study was to determine the effects of seasonal changes in pasture botanical composition on the diet selected by steers grazing rhizoma peanut-mixed tropical grass pastures, as determined by MH and CR analyses.

Materials and Methods

In 1992 and 1993, 11 ruminally cannulated and 78 noncannulated steers were stratified among three "Florigraze" rhizoma peanut-mixed tropical grass (primarily common bermudagrass [Cynodon dactylon] and bahiagrass [Paspalum notatum]) pastures based on BW and visually estimated breed type. Performance for the noncannulated steers was reported by Bennett et al. (1995). Mean shrunk BW at the beginning of the grazing season, March 26, 1992, and March 25, 1993, were 301 \pm 2.8 kg and 288 \pm 3.2 kg, respectively. Even though some steers were removed each year due to the inability to maintain cannulas, each pasture had at least two cannulated steers throughout the grazing season from March 26, 1992, to September 25, 1992 (n = 3, Pasture 1; n = 2, Pasture 2; n = 3, Pasture 3) and March 25, 1993, to September 24, 1993 (n = 2, Pasture 1; n = 2, Pasture 2; n = 2, Pasture 3). Prior to the grazing season, all steers were managed as reported by Bennett et al. (1995).

Crossbred steers to be ruminally cannulated were halter broken through the winter, and ruminal cannulas (#1C rumen cannula with rolled inner flange, Bar Diamond, Parma, ID) were surgically installed at least 7 wk before the grazing season. Surgery was performed by staff veterinarians at the University of Florida, College of Veterinary Medicine using a modified procedure of Turner and McIlwairth (1989). Cannulation procedures as well as the entire experimental protocol were approved by the institutional animal care and use committees of the University of Florida and the USDA, ARS, Subtropical Agricultural Research Station, Brooksville, FL.

Forage Mass, Quality, and Pasture Botanical Composition on Offer

Forage mass and botanical composition (percentage rhizoma peanut, grass, and forb) on offer were sampled every 28 d during both grazing seasons using a double-sampling technique (Ahmed and Bonham, 1982; Ahmed et al., 1983) with a weighted-disc as described by 't Mannetje (1978). At the start of the grazing season, the forb component included a mixture of cool season annual species (*Lepidium virginicum, Geranium carolinianum, Gnaphalium* sp., *Oenothera lanciata*, and *Linaria canadiensis*), but these constituted a minor component of the sward and were not present after about June of each year. The main contributors to the forb component throughout the

grazing season were Mexican tea (*Chenopodium ambrosoides*) and blackberry (*Rubus* sp.), both of which are perennials. Visual observation indicated that blackberry contributed little to the diet of steers, but, at certain times during the grazing season, Mexican tea was readily consumed.

Direct estimates of forage mass and botanical composition were made on clipped samples taken on every 10th discmeter observation. Clipped forage samples were stored at approximately –18°C until separated into grass, rhizoma peanut, forb, and dead material and were then refrozen. Separated samples were removed from the freezer and dried at 50 to 60°C to constant weight. The DM weight of rhizoma peanut, grass, and forb was expressed as a percentage of live (green) material.

Forage quality (CP, DM basis, and in vitro organic matter digestibility [IVOMD]) was evaluated in hand-separated samples (rhizoma peanut, grass, and forb) composited by pasture. Analyses were performed at the University of Florida Forage Evaluation Support Laboratory (Gainesville). Dry matter and OM of samples were determined according to AOAC (1990). In vitro OM digestibility of forage and ingesta samples was evaluated using a modified procedure (Moore and Mott, 1974) of Tilley and Terry (1963), and CP was determined with the procedure described by Gallaher et al. (1975).

Indirect measurements of botanical composition were performed by making visual estimates of the relative weights of forb, grass, and rhizoma peanut as a percentage of live (green) material for each discmeter measurement ($n=80\ to\ 100\ per$ pasture on each date sampled) as described by Ahmed et al. (1983). Forage mass (DM) on offer included live (green) and dead material. Regression equations for estimating forage mass and botanical composition (Ahmed et al., 1983) were generated using the direct values obtained from clipped samples.

Diet Sample Collection

Fecal grab samples and ingesta samples collected using rumen evacuation (cannulated steers) were used for determination of diet botanical composition. Steers grazed 2 wk before the first rumen evacuations were performed. Ingesta samples from one pasture group were collected each day over three consecutive d, every 56 d during the grazing season for a total of four collections per pasture. The procedure used was as described by Lesperance et al. (1960). At sunrise, steers were gathered from the pasture, penned, haltered, and tethered. Fecal grab samples were collected, rumens emptied, and ruminal contents stored in covered, plastic, thermal containers. Steers were returned to the pasture, allowed to graze for approximately 45 min, and then brought back to the pen and tethered again. Ingesta samples were re2254 BENNETT ET AL.

Olson (1991). Ingesta and fecal samples were put into individual plastic containers, labeled, and stored at -18°C until subsequent processing. Original ruminal contents were replaced into the respective steers and steers were returned to their pastures by 0900.

Fecal grab samples were obtained from noncannulated steers every 28 d after March 26, 1992, and March 25, 1993. Fecal grab samples from a subset of 12 steers (n=4 per pasture) were analyzed for botanical composition.

Microhistological Analysis

Microhistological analysis (Holechek and Gross, 1982; Johnson et al., 1983) was used to determine diet botanical composition based on differences microanatomical features of forage particles in ingesta and fecal samples. Ingesta and fecal samples were dried to constant weight at 50 to 60°C and 70 to 80°C, respectively. Samples were ground to pass through a 1-mm screen, and decolorized with 5% (wt/vol) sodium hypochlorite. A .5-g sample was decolorized with 70 (ingesta samples) or 125 mL (fecal samples) of hypochlorite solution for 24 h. Presence of rhizoma peanut, grass, or forb in a microscopic field was recorded for 20 fields per slide, with five slides per sample for a total of 100 observations per sample. Samples were observed at magnification of $125 \times \text{with}$ an inverted light microscope (Axiovert 35, Zeiss, West Germany) using phase contrast microscopy. Relative composition of each sample was calculated using a frequency to density conversion for each forage type as reported by Fracker and Brischle (1944).

Stable Carbon Isotope Ratio Analysis

Dried rhizoma peanut, grass, forb, ingesta, and fecal samples were ground in a Wiley Mill to pass through a 1-mm screen. A 1.5-g subsample of each was then ground to a powder with a Wig-L-Bug (Crescent Dental Mfg. Co., Lyons, IL). Analysis of ¹³C as described by Barrie and Lemley (1989) was performed using an automated N/C analyzer-mass spectrometer (Europa Scientific Ltd., Crewe, England) by a commercial laboratory (Metabolic Solutions, Merrimack, NH). For this analysis, duplicate 1.0-mg samples of the powdered subsamples were sealed in tin capsules, dropped into a combustion tube (Cr₂O₃, 1.020°C), and flash combusted at 1.700°C. Combustion products were drawn into a reduction tube (Cu, 600°C), and magnesium perchlorate and Carbosorb (BDH Ltd., Poole, England) traps removed water and carbon dioxide. Carbon dioxide in the helium carrier gas was analyzed by mass spectrometry.

The ratio of 13 C to 12 C was expressed as the difference (δ) in parts per thousand from the carbon isotope ratio in a carbonate standard (Pee Dee Belemnite). Equations proposed by Coates et al. (1987) were used to predict percentage of C_0 and C_4

plants in the diet using $\delta^{13} \text{C}$ of ingesta or fecal samples.

Statistical Analyses

Pasture forage mass and botanical composition on offer were analyzed as a split-plot in time using the GLM procedure of SAS (1989). When significant, differences among means were separated with the PDIFF option. Main plots were pasture and year and were tested with the pasture \times year interaction. The subplot was sampling date. Sampling date and the interactions with sampling date were tested with the residual error term. The three-way interaction for all botanical components was significant, so effects of sampling date were reanalyzed as a one-way ANOVA by year and pasture using PROC GLM.

Forage quality measurements, CP and IVOMD, were analyzed for effects of year, sampling date, and the year \times sampling date interaction using PROC GLM. The error term for year and sampling date was the interaction term. For this analysis, pasture was the experimental unit.

The relationship between ingesta and fecal botanical components from cannulated steers was evaluated by linear regression using PROC GLM for both MH and CR analyses. Initial analyses included the effect of year as a class variable and the botanical component \times year interaction. When the botanical component \times year interaction was significant, data were reanalyzed by year. For CR, additional analyses were conducted using fecal data corrected for IVOMD of the botanical components (Coates et al., 1987).

The effect of pasture (fixed) and days on pasture (random) on diet botanical composition determined by MH and CR analyses of feces from noncannulated steers was analyzed using PROC GLM. The pasture × days on pasture interaction was used to test the effect of pasture on diet botanical composition. The pasture botanical composition estimated from fecal MH and CR analyses was then compared with the botanical composition of forage on offer with a paired t-test using the MEANS procedure of SAS (1989).

Results and Discussion

Forage Mass, Quality, and Pasture Botanical Composition on Offer

There was a pasture \times year interaction (P < .001) due to greater forage mass in Pasture 1 in 1993 (2.59 \pm .019, 2.47 \pm .019, and 2.45 \pm .019 Mg/ha for Pasture 1, 2, and 3, respectively) than in 1992 (2.45 \pm .018, 2.40 \pm .018, and 2.47 \pm .018 for Pasture 1, 2, and 3, respectively). As would be expected, forage mass on offer was also affected by sample date (P < .001) and its interaction with pasture and year (data not prescribed). Additionally within total forage mass on

(1987) were used to predict percentage of C₃ and C₄ presented). Additionally, within total forage mass on Downloaded from jas.fass.org at USDA Natl Agricultural Library on May 21, 2008.

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offer, there were pasture \times year \times sampling date interactions (P < .001) for the percentage on offer of the three botanical components measured (Figure 1). Changes in both forage mass on offer and botanical composition were due to normal variation among pastures and in seasonal and yearly climate (temperature and rainfall) that affect plant growth. At this location, the growth of tropical species such as rhizoma peanut and bahiagrass is often limited by dry (1992) or cool conditions (1993) in the spring, with rapid growth occurring after the onset of the summer rainy season that occurred in June of 1992 (56 d) and July of 1993 (84 d).

As previously reported by Bennett et al. (1995), however, gains of steers grazing this pastures were never limited by this range or botanical compositon of the herbage mass on offer. When herbage mass is not limiting, the overriding factor that affects an animal's diet selection is the nutritive value of the species

available. There was a sampling date (P < .05) and year \times sampling date effect for both CP and IVOMD. The year \times sampling date interaction was significant for all botanical components except for IVOMD of rhizoma peanut (P = .16) and forb (P = .10). Ranges throughout the season in CP content (28.2 to 12.4% and 16.1 to 7.6% for rhizoma peanut and grass, respectively) and IVOMD (77.0 to 61.2% and 66.3 to 39.1% for rhizoma peanut and grass, respectively) were similar to values reported by Williams et al. (1991).

Relationship Between Ingesta and Fecal Botanical Components

Ingesta and fecal botanical composition from cannulated steers was compared to determine whether ingesta and fecal samples would provide similar information on diet botanical composition. For the MH analyses, there were interactions with year for rhi-

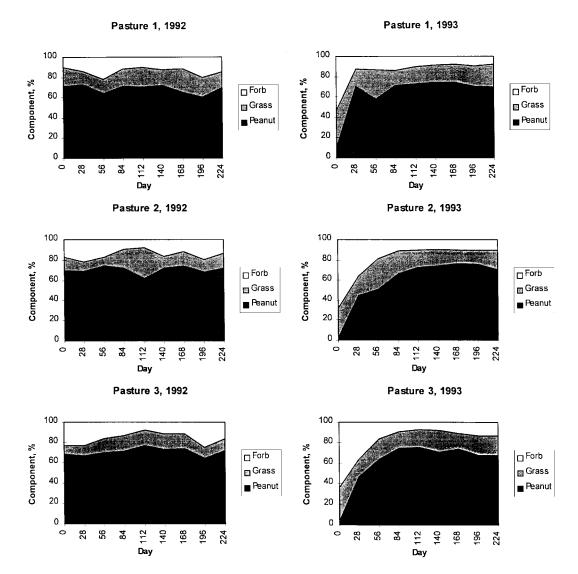


Figure 1. Botanical composition (rhizoma peanut, grass, and forb DM as a percentage of live [green] forage) on offer by pasture, year, and days on pasture (sampling date): pasture × year × day interaction, P < .001.

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2256 BENNETT ET AL.

zoma peanut and forb (P < .05), but not for grass. In 1992 and 1993, ingesta and fecal rhizoma peanut (r = .73 and .92, respectively) and ingesta and fecal forb (r = .86 and .98, respectively) were positively correlated (P < .001). Across years, ingesta and fecal grass were positively correlated (r = .52, P < .001), but the correlation was not as high as for rhizoma peanut or forb. The relatively small range of values for the grass (16 to 29 percentage units for ingesta and 7 to 24 percentage units for feces) over the entire grazing season compared with rhizoma peanut (45 to 80 percentage units for ingesta and 53 to 85 percentage for feces) and forb (33 to 51 for ingesta and 51 to 65 percentage units for feces) probably contributed to this low correlation. Todd and Hansen (1973) found that fecal and ingesta botanical composition were different and concluded that this difference was because fecal samples represented an average of the diet over time. Vavra and Holechek (1980) found that some forage species were sensitive to epidermal destruction during passage throughout the digestive tract, making microhistological characteristics of fecal samples different from extrusal and ruminal samples. Mukhtar and Hansen (1983) found that differential digestion affected discernability of plant fragments, but they concluded that technicians with proper training could identify faintly discernible cuticular fragments. Alipayo et al. (1992) concluded that botanical composition of diets fed were accurately estimated by MH analysis of feces, but there could be a tendency to overestimate legumes and forbs and underestimate grass.

Botanical composition of the selected diet also was determined using stable CR analysis of ingesta and feces. There was no effect of year or botanical component \times year interaction, so these effects were removed from the final analyses. Ingesta and fecal

plants (r = .63, \bar{x} = 87.7%) and ingesta and corrected fecal (corrected for IVOMD) C_3 plants (r = .61, \bar{x} = 87.7%) were positively correlated (P < .001). Even though correction for IVOMD did not improve the correlation between ingesta and fecal C_3 plants, it did move the slope of the relationship closer to unity (from .73 to .96) and moved the intercept closer to zero (from 27.9 to 8.0). Carulla et al. (1991) reported a low correlation (r = .55) between estimates of percentage of legume in the diet determined with $\delta^{13}C$ of feces and $\delta^{13}C$ of esophageal extrusa, and they concluded that this was because botanical composition in feces represents a longer period of consumption than that sampled over a shorter interval in the extrusa.

Diet Selection Estimated from Fecal Microhistological Analysis and Carbon Ratio Analysis vs Pasture Botanical Composition on Offer

Mean differences between botanical components of the herbage on offer and the botanical components of the diet as determined by MH or CR analysis of fecal samples are shown for 1992 in Table 1 and for 1993 in Table 2. Both methods show similar seasonal and yearly effects on diet selection when consideration is given to the fact in CR analysis, rhizoma peanut and forb components cannot be differentiated because they are both C_3 plants.

In 1992, and consistent with the MH analyses, C_4 forage (tropical grass) was selected by steers to a greater extent (P < .05 to P < .01) than grass present in the sward early in the grazing season (d 28 to 56). In 1993, a preference (P < .10 to P < .01) for the grass was not evidenced by MH or CR analysis until d 84 due to limited availability of the grass that year

Table 1. Difference between forage on offer and fecal botanical composition throughout the grazing season estimated by microhistological and carbon ratio analysis in 1992

	Microhistological						Carbon ratio			
Days ^a	Rhizoma peanut		Forb		Grass		C ₃ species		C ₄ species	
	Differenceb	P^{c}	Difference	P	Difference	P	Difference	P	Difference	P
28	-11.5 ± 3.65	*	+7.7 ± 2.55	*	+4.7 ± 2.02	*	-3.4 ± 1.75	†	+4.4 ± 1.75	*
56	-5.6 ± 2.43	*	$+1.6 \pm 1.98$	NS	$+5.0 \pm 1.29$	**	-5.2 ± 1.60	**	$+6.3 \pm 1.60$	**
84	2 ± 2.98	NS	$+2.8 \pm 1.60$	NS	-1.6 ± 2.14	NS	$+3.6 \pm 3.09$	NS	-2.6 ± 3.11	NS
112	$+19.5 \pm 3.85$	***	-3.3 ± 1.09	*	-15.1 ± 3.01	***	$+11.3 \pm 3.48$	**	-10.3 ± 3.48	*
140	$+18.4 \pm 1.71$	***	-8.7 ± 1.04	***	$-8.7 \pm .89$	***	$+2.5 \pm 1.30$	†	-1.5 ± 1.29	NS
168	$+20.3 \pm 2.37$	***	$-9.5 \pm .37$	***	-9.7 ± 2.17	**	8 ± 1.52	NS	$+1.8 \pm 1.52$	NS
196	$+27.2 \pm 1.34$	***	$-19.0 \pm .75$	***	-7.2 ± 1.55	***	-16.1 ± 2.62	***	$+17.1 \pm 2.62$	***
224	$+19.3 \pm 2.57$	***	$-11.9 \pm .68$	***	-6.5 ± 2.23	*	$-10.2 ~\pm~ 2.24$	***	$+11.2 ~\pm~ 2.24$	***

^aDays on pasture with d 0 = March 26, 1992.

bMean difference expressed in percentage units; (+) = forage component was more abundant in estimated diet than in forage on offer; (-) = forage component was less abundant in estimated diet than in forage on offer.

^cProbability the mean difference is different from 0: NS = P > 10: * = P < 05: ** = P < 11: ** = P < .001.

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Table 2. Difference between forage on offer and fecal botanical composition throughout the grazing season estimated by microhistological and carbon ratio analyses in 1993

	Microhistological						Carbon ratio				
Days ^a	Rhizoma peanut		Forb		Grass		C ₃ species		C ₄ species		
	Differenceb	P^{c}	Difference	P	Difference	P	Difference	P	Difference	P	
28	-9.6 ± 2.43	**	+14.5 ± 2.91	***	-4.8 ± 1.67	*	+4.0 ± 2.77	NS	-4.0 ± 2.78	NS	
56	$+7.9 \pm 2.47$	**	$+4.1 \pm 2.47$	NS	-12.0 ± 1.54	***	$+11.5 \pm 2.05$	***	-11.5 ± 2.05	***	
84	$+2.7 \pm 2.37$	NS	$-6.7 \pm .86$	***	$+4.0 \pm 2.03$	†	$-10.5~\pm~2.51$	**	$+10.5 \pm 2.51$	**	
112	$+14.0 \pm 1.53$	***	$-7.8 \pm .33$	***	-6.2 ± 1.39	**	$+1.2 \pm 1.53$	NS	-1.2 ± 1.53	NS	
140	$+17.4 \pm 2.32$	***	-5.1 ± 1.23	**	$-12.4~\pm~1.26$	***	$+8.3 \pm 1.53$	***	-8.3 ± 1.53	***	
168	$+15.9 \pm 1.05$	***	$-8.7 \pm .60$	***	-7.2 ± 1.01	***	-1.9 ± 1.45	NS	$+1.9 \pm 1.45$	NS	
196	$+24.6 \pm 1.25$	***	$-10.7 \pm .54$	***	$-14.1 \pm .95$	***	$+5.5 \pm 1.06$	***	-5.6 ± 1.06	***	
224	$+24.5 \pm .87$	***	$-9.5 \pm .88$	***	$-15.0 \pm .72$	***	$+5.9~\pm~1.15$	***	$-6.0~\pm~1.14$	***	

^aDays on pasture with d 0 = March 25, 1993.

(Figure 1), the result of a late April frost. Stobbs (1977) found that a legume, siratro (*Macroptilium atropurpureum*), grown in association with tropical grass, contributed little to the diet of grazing cattle in spring and early summer. However, he attributed these results in part to low yield of legume relative to grass during spring, which did not seem to be the case for rhizoma peanut in the present experiment, at least in 1992 (Figure 1).

For both years, by d 112, CR analysis showed that intake of C_4 forage declined (P < .05), and this was consistent with the MH analysis. This was due to relatively greater (P < .01) intake of C_3 plants (rhizoma peanut and forb). Microhistological analysis showed that this increase in C₃ plants was composed almost exclusively of rhizoma peanut and not forb. At this time, the forb component was mostly Mexican tea, which the animals avoided. Stobbs (1977) in Australia and Carulla et al. (1991) in the Llanos of Colombia showed that cattle had a preference for legume in mixed swards later in the grazing season. Langlands and Sanson (1976) concluded that selectivity increased as bulk density of the forage on offer increased. This was attributed to a decline in nutritive value of forage on offer as forage mass increased, offering more opportunity for cattle to selectively graze. The present data seem to be consistent with these observations because selection for rhizoma peanut started to occur when forage mass on offer increased due to summer rains.

For the remainder of the grazing season, there were discrepancies between CR and MH analysis (Table 1 and 2). Carbon ratio analysis indicated that C_3 components were either not selected by steers or C_4 in the diet was significantly greater and C_3 in the diet significantly less than that on offer. This is in contrast to the work of Coates et al. (1987), who used CR analysis to study diets of cattle grazing grass-legume

pastures that consisted mainly of rhodesgrass (*Chloris gayana* Kunth.) and stylo (*Stylosanthes hamata* [L.] Taub. and *viscosa* Sw.) in a subtropical region of Australia, south of Townsville, Queensland. They found that preference for legume was high for much of the study, but it was highest during fall. The apparent discrepancy between MH and CR analyses (Table 1 and 2) during this time period is likely due to the combined effect of steers avoiding forb and selecting rhizoma peanut, both of which are C₃ plants.

In conclusion, we found that fecal botanical composition was correlated with botanical composition of ingesta, and, therefore, fecal botanical composition may be useful in determining diet selection. An advantage of this approach (fecal analysis) would be the elimination of surgery required to install ruminal or esophageal cannulas. Results from two methods of determining botanical composition in feces, MH and CR, were different at times due to the complexity of the sward (both rhizoma peanut and forb constituted a single component in the CR analysis). In such cases, MH analysis may be the method of choice. When the C₃ component is primarily the legume(s) of interest, CR analysis may be advantageous because it eliminates substantial technical skill required to perform MH analysis.

Implications

Both microhistological and carbon ratio analysis of feces can be used to estimate diet botanical composition in cattle grazing mixed swards of tropical grasses and legumes. If there is substantial contribution of forb to the sward, such as occurred with *Chenopodium ambrosoides* in the present experiment, fecal microhistological analysis may be more informative than fecal carbon ratio analysis.

 $^{^{}b}$ Mean difference expressed in percentage units; (+) = forage component was more abundant in estimated diet than in forage on offer; (-) = forage component was less abundant in estimated diet than in forage on offer.

Probability the mean difference is different from 0; NS = P > .10; $\uparrow = P < .10$; * = P < .05; ** = P < .01; *** = P < .001.

Literature Cited

- Ahmed, J., and C. D. Bonham. 1982. Optimum allocation in multivariate double sampling for biomass estimation. J. Range Manage. 35:777–779.
- Ahmed, J., C. D. Bonham, and W. A. Laycock. 1983. Comparison of techniques used for adjusting biomass estimates by double sampling. J. Range Manage. 36:217–221.
- Alipayo, D., R. Valdez, J. L. Holchek, and M. Cardenas. 1992. Evaluation of microhistological analysis for determining ruminant diet botanical composition. J. Range Manage. 45: 148–152.
- AOAC. 1990. Official Methods of Analysis (15th Ed.). Association of Official Analytical Chemists, Arlington, VA.
- Barrie, A., and M. Lemley. 1989. Automated 15N/13C analysis of biological materials. Am. Lab. 21:54–63.
- Bennett, L. L., A. C. Hammond, M. J. Williams, W. E. Kunkle, D. D. Johnson, R. L. Preston, and M. F. Miller. 1995. Performance, carcass yield, and carcass quality characteristics of steers finished on rhizoma peanut (*Arachis glabrata*) –tropical grass pasture or concentrate. J. Anim. Sci. 73:1881–1887.
- Carulla, J. E., C. E. Lascano, and J. K. Ward. 1991. Selectivity of resident and oesophageal fistulated steers grazing *Arachis pin*toi and *Brachiaria dictyoneura* in the Llanos of Columbia. Trop. Grassl. 25:317–324.
- Coates, D. B., P. Schachenmann, and R. J. Jones. 1987. Reliability of extrusa samples collected from steers fistulated at the oesophagus to estimate the diet of resident animals in grazing experiments. Aust. J. Exp. Agric. 27:739–745.
- Coleman, S. W., and K. M. Barth. 1973. Quality of diets selected by grazing animals and its relation to quality of available forage and species composition of pastures. J. Anim. Sci. 36:754–761.
- Fracker, S. B., and H. A. Brischle. 1944. Measuring the local distribution of ribes. Ecology 25:283–303.
- Gallaher, R. M., C. O. Weldon, and J. G. Futral. 1975. An aluminum digester for plant and soil analysis. Soil Sci. Soc. Am. Proc. 39: 803–806.
- Holechek, J. L., and B. Gross. 1982. Evaluation of different calculation procedures for microhistological analysis. J. Range Manage. 35:721–723.
- Johnson, M. K., H. Wofford, and H. A. Pearson. 1983. Microhistological techniques for food habits analyses. Res. Paper SO-199. USDA, Forest Service, Southern Forest Exp. Sta., New Orleans, LA.
- Jones, R. J., M. M. Ludlow, J. H. Troughton, and C. G. Blunt. 1979. Estimation of the proportion of C_3 and C_4 plant species in the diet of animals from the ratio of natural $^{12}\mathrm{C}$ and $^{13}\mathrm{C}$ isotopes in the faeces. J. Agric. Sci. (Camb.) 92:91–100.
- Langlands, J. P., and J. Sanson. 1976. Factors affecting the nutritive value of the diet and the composition of rumen fluid of grazing sheep and cattle. Aust. J. Agric. Res. 27:691–707.

- Lesperance, A. L., E. H. Jensen, V. R. Bohman, and R. A. Madsen. 1960. Measuring selective grazing with fistulated steers. J. Dairy Sci. 43:1615–1622.
- Minson, D. J., M. M. Ludlow, and J. H. Troughton. 1975. Differences in natural carbon isotope ratios of milk and hair from cattle grazing tropical and temperate pastures. Nature (Lond.) 256: 602.
- Moore, J. E., and G. O. Mott. 1974. Recovery of residual organic matter from in vitro digestion of forages. J. Dairy Sci. 57: 1258–1259.
- Moore, J. E., L. E. Sollenberger, G. A. Morantes, and P. T. Beede.
 1987. Canopy structure of *Aeschynomene americana-Hemarthria altissima* pastures and ingestive behavior of cattle. In: F. P. Horn, J. Hodgson, J. J. Mott, and R. W. Brougham (Ed.)
 Grazing Lands Research at the Plant Animal Interface. pp
 93–98. Winrock International, Morrilton, AR.
- Mukhtar, H. K., and R. M. Hansen. 1983. Fiber effects on microhistological analysis. J. Range Manage. 36:477–478.
- Olson, K. C. 1991. Diet sample collection by esophageal fistula and rumen evacuation techniques. J. Range Manage. 44:515–519.
- SAS. 1989. SAS/STAT® User's Guide (Version 6, 4th Ed.). SAS Inst. Inc., Cary, NC.
- Sparks, D. R., and J. C. Malechek. 1968. Estimating percentage dry weight in diets using a microscope technique. J. Range Manage. 21:264–265.
- Stobbs, T. H. 1977. Seasonal changes in the preference by cattle for Macroptilium atropurpureum cv. siratro. Trop. Grassl. 11: 87-91.
- Tilley, J.M.A., and R. A. Terry. 1963. A two-stage technique for the in vitro digestibility of tropical grasses. J. Br. Grassl. Soc. 18: 104–111.
- Todd, J. W., and R. M. Hansen. 1973. Plant fragments in the feces of bighorns as indicators of food habits. J. Wildl. Manage. 37: 363–366.
- 't Mannetje, L. 1978. Measuring quantity of grassland vegetation. In: L. 't Mannetje (Ed.) Measurement of Grassland Vegetation and Animal Production, Bull. 52. pp 63–95. Commonwealth Bureau of Pastures and Field Crops, Hurley, Berkshire, England.
- Turner, A. S., and C. McIlwairth. 1989. Techniques in Large Animal Surgery. Lea and Febiger, Philadelphia, PA.
- Varva, M., and J. L. Holechek. 1980. Factors influencing microhistological analysis of herbivore diets. J. Range Manage. 33: 371–374.
- Williams, M. J., A. C. Hammond, W. E. Kunkle, and T. H. Spreen. 1991. Stocker performance on continuously grazed mixed grass-rhizoma peanut and bahiagrass pastures. J. Prod. Agric. 4: 19–24.